J. Raptor Res. 33(2):128–133

METHODS FOR GENDER DETERMINATION OF CRESTED CARACARAS

JOAN L. MORRISON[1]
Department of Wildlife Ecology and Conservation, P.O. Box 110430, University of Florida, Gainesville, FL 32611 U.S.A.

MARY MALTBIE[2]
Department of Biological Sciences, Texas Tech University, Box 43131, Lubbock, TX 79409-3131 U.S.A.

ABSTRACT.—We report details of a method that is reliable for gender determination of Crested Caracaras (*Caracara plancus*). Using the microsatellite probe Poly(dA-dG)·(dC-dT), we detected sex-specific (female only) high-molecular weight restriction fragments in DNA from blood samples collected in the field. This method correctly identified the gender of 14 known-sex captive caracaras and was subsequently used to identify gender for 28 wild adults. We also evaluated morphometric measurements for these 42 individuals to determine whether any single characteristic or combination could be used in the field to make reliable gender determinations. No morphometric measurements were found that were reliable for gender determination in adult caracaras. Bill depth and wing length tended to be larger for females, but there was considerable overlap among the sexes for all measurements. Bill depth and wing length were retained in a model developed using multiple logistic regression analysis, but the model's overall predictive capability for indicating gender was poor. Because young caracaras are only 80% of adult size at fledging, their morphometric measurements are not usable for gender determination. Genetic methods are likely the only reliable methods suitable for determining gender in Crested Caracaras.

KEY WORDS: *Crested Caracara*; Caracara plancus; *DNA*; *blood sampling*; *gender determination.*

Metodos para la determinacion de genero de *Caracara plancus*

RESÚMEN.—Reportamos detalles de un método confiable para la determinación de género de *Caracara plancus*. Mediante la utilización de la exploración de microsatélite Poli (dA-dG)·(dC-dT) detectamos altos fragmentos moleculares de peso restringidos (en hembras solamente) en el ADN proveniente de muestras tomadas en campo. Este método identificó en forma correcta el género de 14 caracaras cautivos de los cuales se conocía el sexo. Subsecuentemente fué utilizado para la identificación de 28 adultos silvestres. También evaluamos las medidas morfométricas de estos 42 individuos para determinar si alguna característica o combinación puede ser usada en campo para la determinación de género. Ninguna medición morfométrica fué confiable para la identificación de género en caracaras adultos. La profundidad del pico y la longitud del ala tendían a ser mayores en las hembras, hubo coincidencias en las medidas de ambos sexos. La profundidad del pico y la longitud del ala fueron utilizados para la aplicación de un análisis de regresión multiple, pero en general la capacidad predictiva del modelo para la identificación del género fué pobre. Debido a que los caracaras jóvenes son un 80% del tamaño adulto cuando son pichones, sus medidas morfométricas no son utilizables para la determinacion de género. Los métodos genéticos parecen ser los únicos confiables para determinar el género de los caracaras crestados.

[Traducción de César Márquez]

The ability to identify the gender of birds is critical to ecological studies for addressing a variety of topics including population and brood sex ratios, sex-ratio manipulation and gender-related differences in dispersal, habitat use, site fidelity, and survival. However, gender determination can be difficult for young birds and sexually monomorphic species. Discriminant function and logistic regression analyses have been used along with morphometric data to determine gender for breeding adults in a variety of avian species (Edwards and

[1] Present address: Department of Biology, Colorado State University, Fort Collins, CO 80523 U.S.A.
[2] Present address: Life Sciences Division, Mailstop M888, Los Alamos National Laboratory, Los Alamos, NM 87545 U.S.A.

Kochert 1987, Clark et al. 1991, Smith and Wiemeyer 1992), but rates of misclassification can exceed 20%, particularly for species with considerable overlap in measurements, and these methods cannot be used on young individuals. Hormone immunoassays and genetic methods such as karyotyping and flow cytometry have been used for sexing birds but with varying success (Tiersch et al. 1991) and only to a limited extent for raptors (Ivins 1975). The usefulness and reliability of micro- and minisatellite probes for gender identification in birds, including members of Falconiformes, has been well-documented (Longmire et al. 1991, 1993, Epplen et al. 1991, Delehanty et al. 1995, Fleming et al. 1996).

The Crested Caracara (*Caracara plancus*) is a little-known tropical raptor with a limited distribution in North America. Although distinct plumage differences can be recognized among age groups (Wheeler and Clark 1995, Morrison 1996), there are no distinguishable gender-related plumage differences. Snyder and Wiley (1976) reported a low index of dimorphism (2.2) for this species, and considerable overlap in measurements exists between males and females for all subspecies (Morrison 1996). Both males and females incubate, so both have a brood patch (Morrison 1996).

In Florida, the Crested Caracara occurs as an isolated population in the southcentral peninsula (Stevenson and Anderson 1994). Because of its small size, restricted range, and apparent vulnerability to habitat changes, this population is listed as Threatened by the U.S. Fish and Wildlife Service and by the state of Florida. Recent studies on the ecology and dynamics of this population have been limited by the inability to determine the gender of individuals.

The objective of this study was to identify techniques suitable for gender determination in Crested Caracaras. We investigated the feasibility of using genomic DNA obtained from blood samples to look for the presence of high-molecular weight, female-specific, microsatellite fragments, following Longmire et al. (1993). We also examined external morphological characteristics for known-sex individuals. Reliable gender determination using genetic methods would facilitate our ability to evaluate the use of morphometric measurements for gender determination in the field. Although surgical examination also provides gender information, this was not a viable option for this study because of the cost, difficulties of use in the field, lack of usefulness for sexing juveniles and concern regarding use of an invasive technique on a Threatened Species.

STUDY AREA AND METHODS

We studied Crested Caracaras in southcentral Florida (27°10′N, 81°12′W). Nesting territories were located in eight counties: Highlands, Glades, Okeechobee, Osceola, DeSoto, Polk, Hendry, and Indian River. This region constitutes most of the species' current breeding range in Florida.

During 1994–96, we took blood samples and morphometric measurements from 42 Crested Caracaras, including 14 captives and 28 wild, breeding adults that were captured throughout the study area (Morrison and McGehee 1996). Using laparoscopy, we determined gender of the 14 captive individuals, which were subsequently used for genetic gender determination.

We used blood samples obtained from the 14 known-sex captive caracaras (5 M, 9 F) to identify a suitable probe for gender identification. Approximately 0.2 μl of blood was collected from a brachial vein of the wing and transferred immediately into 5 ml of lysis buffer (Arctander 1988). Samples were sent to the laboratory for processing without information on the known gender of the captive individuals.

Genomic DNA was isolated using a modified procedure from Longmire et al. (1991). The concentration of each DNA sample was estimated using a UV spectrophotometer. Approximately 10 μg of DNA from each sample was digested with the enzymes *Hae* III and *Hinf* I in two separate reactions using reaction conditions recommended by the supplier (New England Biolabs, Beverly, MA U.S.A.). Digested samples were electrophoresed in a 0.8% agarose gel at 40 volts for 36 hr. Restriction fragments were transferred to positively charged nylon membranes (Amersham, Arlington Heights, IL U.S.A.) using a modification of Southern (1975). Membranes were then baked for 2 hr at 65°C. Hybridization procedures followed the protocol of Longmire et al. (1993) except that prehybridization and hybridization solutions were 6 × SSC, 0.01 M EDTA pH 8.0, 10 × Denhardts solution and 1% (w/v) SDS. The probe used to identify gender was Poly(dA-dG)·(dC-dT) (supplied by Pharmacia, Piscataway, NJ U.S.A.). Post hybridizations, membranes were washed twice for 5 min each in 2 × SSC, 0.1% SDS at 22°C and twice for 5 min each in 0.05 M NaCl, 0.1% SDS at 42°C. Membranes were then exposed to film (Amersham Hyperfilm MP) overnight in cassettes with two intensifying screens. Radiographs were examined for presence of gender-specific high-molecular-weight bands (Longmire et al. 1993).

Blood samples from wild caracaras were collected and processed for gender determination, as above. We determined gender of the 28 wild caracaras by applying the genetic method after it was validated using the 14 captives.

We took five external morphometric measurements on all 42 Crested Caracaras. The captive individuals had originally come from the wild as either immatures or adults, so we assumed that their measurements were not different (due to being in captivity) from those of other

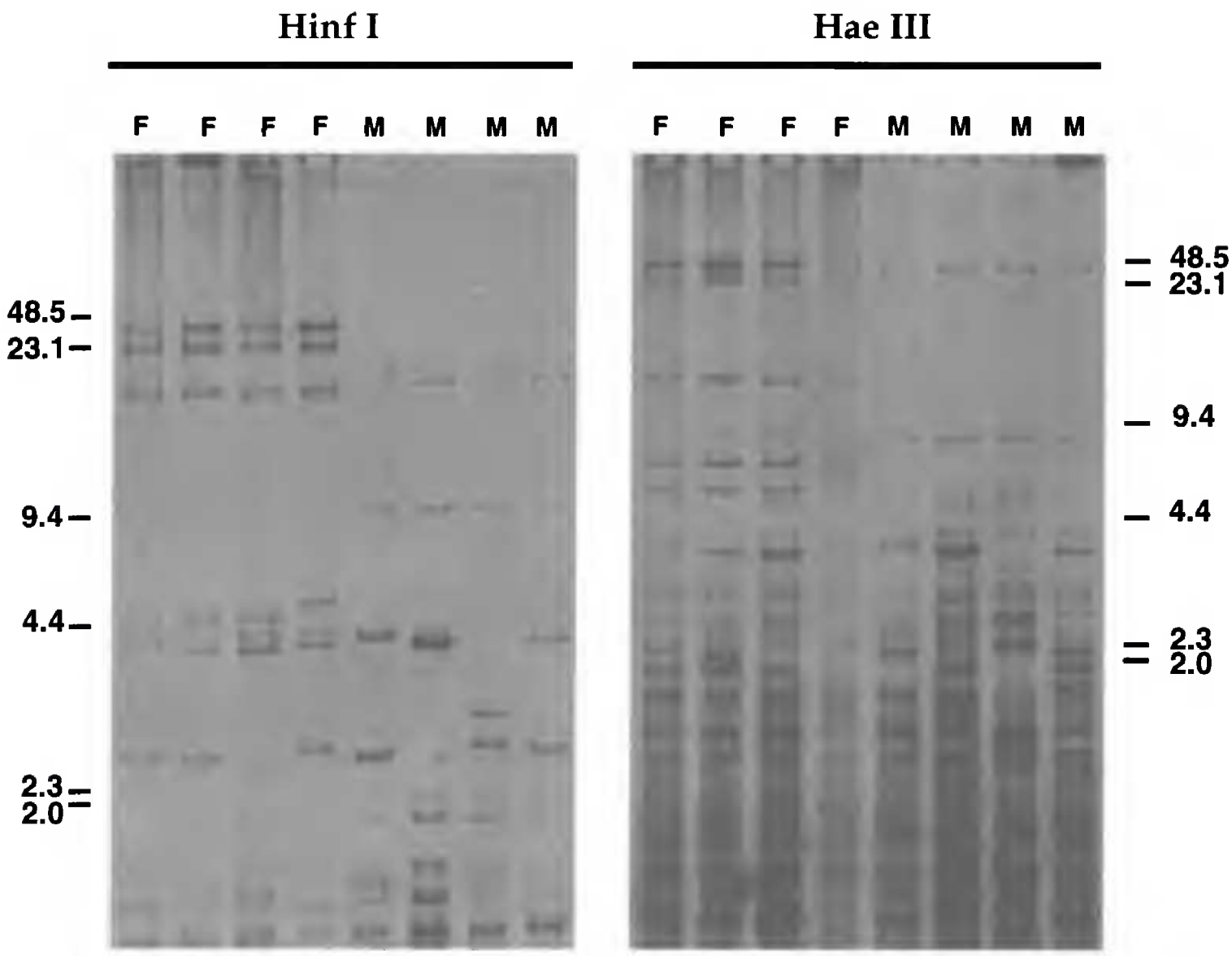

Figure 1. Representative autoradiograms of four female (F) and four male (M) Crested Caracaras. Genomic DNA was digested with the enzymes *Hinf* I (left panel) and *Hae* III (right panel). Both sets of digestions are hybridized with the probe Poly(dA-dG)·(dC-dT). To the side of each autoradiogram are the molecular weight markers in kilobases.

wild individuals. Four of the 14 (1 M, 3 F) were in Basic I plumage (Wheeler and Clark 1995) so were at least one year old. We assumed their measurements were not different from those of adults (Morrison unpubl. data).

Measurements followed the North American Bird Banding Techniques Manual (1984). All the following were taken in mm: wing length (length of the unflattened wing from the bend to the tip of the longest primary), culmen (from the cere edge to the tip of the bill), tarsus length (back of the intertarsal joint to the lower edge of the last complete scale before the toes), bill depth (cere edge to the bottom of the lower mandible at the deepest point), and bill width (maximum measurement at the posterior part of the bill). We did not measure tails because we considered this measurement unreliable. Caracaras regularly walk on the ground while foraging, and their tails incur considerable wear and breakage distally. Mass was not considered reliable because many captured wild individuals had engorged crops.

We compared measurements ($\bar{x} \pm 1$ SE) between males and females for all 42 caracaras using unpaired, 2-tailed *t*-tests. We also used multiple logistic regression (MLR) on the entire set of measurements. MLR relates several explanatory variables, in this case morphological measurements, to a dichotomous dependent variable, in this case, gender. MLR is more appropriate than discriminant function analysis for these comparisons, particularly when assumptions of multivariate normality are violated (Press and Wilson 1978). The multiple logistic function is the probability of an individual belonging to one particular group, in this case, the probability of any individual being female. Probabilities below a threshold value (assigned as 0.50) indicate male and higher probabilities indicate female. MLR was conducted using SigmaStat ver. 2.0 (Jandel Scientific, Inc. 1995).

RESULTS

Genetic Method. We correctly identified the gender of all 14 captive caracaras using the microsatellite probe. All females ($N = 9$) exhibited 2 fragments larger than 23 kb. All males exhibited only a single band in this same size range (Fig. 1). Thus, female caracaras were distinguishable by the presence of another band at or in excess of 23 kb, in comparison to males, which did not show this second band. In the *Hae* III digested samples, the

Table 1. Comparison of five external morphological characteristics of 42 (14 captive and 28 wild) Crested Caracaras in southcentral Florida, 1994–96. All measurements in mm.

	MALE (N = 23)			FEMALE (N = 19)				
CHARACTERISTIC	MEAN	SE	RANGE	MEAN	SE	RANGE	t	P
Wing length	392.41	2.99	345.0–408.0	404.68	2.64	384.0–430.0	3.07	0.004
Tarsus	103.77	0.45	98.6–107.8	103.89	0.98	94.6–113.0	0.18	0.91
Culmen	33.12	0.20	31.5–35.0	33.56	0.55	24.6–35.6	0.74	0.46
Bill depth	23.56	0.13	22.5–24.9	24.2	0.17	22.9–25.6	2.97	0.005
Bill width	13.86	0.13	12.2–15.0	14.04	0.12	13.0–15.0	0.98	0.35

marker was above 23 kb. The marker in the *Hinf* I digested samples started at about 23 kb (Fig. 1).

Morphometric Analyses. Only bill depth and wing length differed between males and females (Table 1), though considerable overlap was noted even for these two characteristics (Table 2). Only bill depth and wing length were retained in the logistic regression model: probability of being female = −55.79 + (0.064*wing length) + (1.263*bill depth) (χ^2 = 40.81, $P < 0.001$). Predictive capability of the model was poor, however, resulting in a mean misclassification rate of 59%. Most females were correctly identified but 86% of males were incorrectly classified as females.

Table 2. Percentage of male and female Crested Caracaras in each measurement group for wing length and bill depth. All measurements in mm.

WING LENGTH	NO. OF MALES	%	NO. OF FEMALES	%
345–350	2	0.09	0	0.00
386–390	6	0.26	2	0.10
391–395	4	0.17	2	0.10
396–400	7	0.30	3	0.16
401–405	1	0.04	2	0.10
406–410	3	0.13	4	0.21
411–415	0	0.00	3	0.16
416–420	0	0.00	3	0.16
Total	23		19	
BILL DEPTH	NO. OF MALES	%	NO. OF FEMALES	%
22.5–23.0	5	0.22	1	0.05
23.1–23.5	6	0.26	3	0.16
23.6–24.0	8	0.35	5	0.26
24.1–24.5	2	0.09	4	0.21
24.6–25.0	2	0.09	2	0.11
25.1–25.5	0	0.00	3	0.16
25.6–26.0	0	0.00	1	0.05
Total	23		19	

DISCUSSION

Genetic analyses correctly identified the gender of all known-sex Crested Caracaras. This technique can also be used for gender determination in hatch-year (HY) and after hatch-year (AHY) caracaras. Even at fledging, young caracaras cannot be sexed reliably using morphometric measurements because they are only approximately 80% of overall adult size (Morrison 1996).

A variety of techniques have been used for gender identification in birds, reviewed by Ellegren and Sheldon (1997). Recently, a set of universal primers were published that will identify gender in all groups of birds except for ratites (Griffiths et al. 1996). Use of these universal primers would have been the technique of choice if we had sought only gender information for individual caracaras. An advantage of using microsatellite fingerprint analysis for gender identification is that these fingerprint patterns can also be used to examine population-level parameters such as genetic diversity within the study group. Membranes obtained from our analyses can be rehybridized with other mini- and microsatellite probes to obtain information on the frequency of polymorphic fragments. Hypervariable DNA fragment patterns have been successfully used in other population studies of birds (Longmire et al. 1991, 1992).

We did not detect any adverse effects of blood sampling and handling procedures on sampled individuals. Because adults were year-round residents within their territories, all individuals sampled were resighted numerous times following handling and blood sampling. We also sampled over 100 HY caracaras that were fitted with radiotransmitters. The resighting rate at independence (2 months

postfledging) for these individuals was 100%. Even nestlings that were sampled at 4–6 wk of age did not appear to incur any adverse effects of the sampling procedures.

Although the caracara does exhibit some slight sexual dimorphism in external characteristics, morphometric measurements proved to be unreliable indicators of gender even for adults. Larger overall size, particularly larger wing length and bill depth, was generally indicative of females. Considerable variation and overlap existed among all characters measured, however, so reliable gender identification of birds in the field from observation alone is not possible. Because of the multivariate nature of these differences, no single combination of characters was found that could reliably predict gender in breeding adults.

ACKNOWLEDGMENTS

We thank S. McGehee, V. Dreitz, L. Todd, and C. Pages for assistance with fieldwork. B. Mealey and B. Bowen provided blood sampling supplies. We thank R. Collins, B. Mealey, G. Straight, D. Wrede, and K. Wrede for permitting us to take blood samples from their captive caracaras. We are grateful for logistic support from the MacArthur Agro-Ecology Research Center of Archbold Biological Station; the Department of Wildlife Ecology and Conservation, the Florida Agricultural Experiment Station, and the Institute of Food and Agricultural Sciences at the University of Florida; R.J. Baker at Texas Tech University; and J.L. Longmire at Los Alamos National Laboratory. Comments from 2 anonymous reviewers improved the manuscript. Most importantly, we thank landowners throughout southcentral Florida, who generously provided access to their lands. This research was funded by the Nongame program of the Florida Game and Fresh Water Fish Commission, the Florida Ornithological Society, the Avon Park Air Force Range, the South Florida Water Management District, and the U.S. Fish and Wildlife Service. This is MacArthur Agro-Ecology Research Center contribution No. 44 and Florida Agricultural Experiment Station journal series R-06604.

LITERATURE CITED

ARCTANDER, P. 1988. Comparative studies of avian DNA by restriction fragment length polymorphism analysis: convenient procedures based on blood samples from live birds. *J. Ornithol.* 129:205–216.

CLARK, R.G., P.C. JAMES AND J.B. MORARI. 1991. Sexing adult and yearling American Crows by external measurements and discriminant analysis. *J. Field Ornithol.* 62:132–138.

DELEHANTY, D.J., R.A. TYBIE, M.J. DITSWORTH, B.A. HOELZER, L.W. ORING AND J.L. LONGMIRE. 1995. Genetic and morphological methods for gender identification of Mountain Quail. *J. Wildl. Manage.* 59:785–789.

EDWARDS, T.C., JR. AND M.N. KOCHERT. 1987. Use of body weight and length of footpad as predictors of sex in Golden Eagles. *J. Field Ornithol.* 58:144–147.

ELLEGREN, H. AND B.C. SHELDON. 1997. New tools for sex identification and the study of sex allocation in birds. *Trends Ecol. Evol.* 12:255–259.

EPPLEN, J.T., H. AMMER, C. EPPLEN, C. KAMMERBAUER, R. MITREITER, L. ROEWER, W. SCHWAIGER, V. STEIMLE, H. ZISCHLER, E. ALBERT, A. ANDREAS, B. BEYERMANN, W. MEYER, J. BUITKAMP, I. NANDA, M. SCHMID, P. NYRNBERG, S. PENA, H. PSCHE, W. SPRECHER, M. SCHARTL, K. WEISING AND A. YASSOURIDIS. 1991. Oligonucleotide fingerprinting using simple repeat motifs: a convenient, ubiquitously applicable method to hypervariability for multiple purposes. Pages 50–69 *in* T. Burke, G. Dolf, A. Jeffreys and R. Wolff [EDS.], DNA fingerprinting: approaches and applications. Birkhauser Press, Brasil, Switzerland.

FLEMING, T.L., J.L. HALVERSON AND J.B. BUCHANAN. 1996. Use of DNA analysis to identify sex of Northern Spotted Owls (*Strix occidentalis caurina*). *J. Raptor Res.* 30: 118–122.

GRIFFITHS, R., S. DAAN AND C. DIJKSTRA. 1996. Sex identification in birds using two CHD genes. *Proc. R. Soc. Lond. Ser. B,* 263:1249–1254.

IVINS, G.K. 1975. Sex determination in raptorial birds—a study of chromatin bodies. *J. Zool. Anim. Med.* 6:9–11.

JANDEL SCIENTIFIC. 1995. SigmaStat version 2.0. Jandel Scientific, San Rafael, CA U.S.A.

LONGMIRE, J.L., R.E. AMBROSE, N.C. BROWN, T.J. CADE, T. MAECHTLE, W.S. SEEGAR, F.P. WARD AND C.M. WHITE. 1991. Use of sex-linked minisatellite fragments to investigate genetic differentiation and migration of North American populations of the Peregrine Falcon (*Falco peregrinus*). Pages 217–229 *in* T. Burke, G. Dolf, A. Jeffreys and R. Wolff [EDS.], DNA fingerprinting: approaches and applications. Birkhauser Press, Brasil, Switzerland.

———, G.F. GEE, C.L. HARDEKOPF AND G.A. MARK. 1992. Establishing paternity in Whooping Cranes (*Grus americana*) by DNA fingerprint analysis. *Auk* 109:522–529.

———, M. MALTBIE, R.W. PAVELKA, L.M. SMITH, S.M. WITTE, O.A. RYDER, D.L. ELLSWORTH AND R.J. BAKER. 1993. Gender identification in birds using microsatellite DNA fingerprint analysis. *Auk* 110:378–381.

MORRISON, J.L. 1996. Crested Caracara. *In* A. Poole and F. Gill [EDS.], The birds of North America 249. Acad. Natl. Sciences, Philadelphia, PA and Am. Ornithol. Union, Washington, DC U.S.A.

——— AND S.M. MCGEHEE. 1996. Capture methods for Audubon's Crested Caracara. *J. Field Ornithol.* 67:630–636.

NORTH AMERICAN BIRD BANDING TECHNIQUES MANUAL. 1984. Canadian Wildlife Service Publ. Vol. 2. Ottawa, Ontario, Canada.

PRESS, S.J. AND S. WILSON. 1978. Choosing between logis-

tic regression and discriminant analyses. *J. Amer. Statistical Assoc.* 73:699–705.

SMITH, D.G. AND S.N. WIEMEYER. 1992. Determining sex of Eastern Screech-Owls using discriminant function analysis. *J. Raptor Res.* 26:24–26.

SNYDER, N.F. AND J.W. WILEY. 1976. Sexual size dimorphism in hawks and owls of North America. *Ornithol. Monogr.* 20:1–96.

SOUTHERN, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503–527.

STEVENSON, H.M. AND B.H. ANDERSON. 1994. The birdlife of Florida. Univ. Press Florida, Gainesville, FL U.S.A

TIERSCH, T.R., R.L. MUMME, R.W. CHANDLER AND D. NAKAMURA. 1991. The use of flow cytometry for rapid identification of sex in birds. *Auk* 108:206–208.

WHEELER, B.K. AND W.S. CLARK. 1995. A photographic guide to North American raptors. Academic Press, San Diego, CA U.S.A.

Received 17 May 1998; accepted 2 February 1999